Biotechnology Reports 30 (2021) e00634

Contents lists available at ScienceDirect

Biotechnology Reports

journal homepage: www.elsevier.com/locate/btre

Short Communication

Genome sequence of the epiphytic bacteria *Bacillus altitudinis* strain 19_A, isolated from the marine macroalga *Ulva lactuca*

Natalia Beatriz Comba González[a], Dolly Montoya Castaño[b], José Salvador Montaña Lara[a,*]

[a] Unidad de Investigaciones Agropecuarias (UNIDIA), Departamento de Microbiología, Pontificia Universidad Javeriana, Bogotá, Colombia
[b] Grupo de Bioprocesos y Bioprospección, Instituto de Biotecnología, Universidad Nacional de Colombia, Bogotá, Colombia

ARTICLE INFO

Article history:
Received 28 April 2020
Received in revised form 16 May 2021
Accepted 17 May 2021

Keywords:
Genome
Enzymes
Bacillus altitudinis
Macroalgae

ABSTRACT

Microorganisms living on marine macroalgal surfaces require enzyme repertoires to metabolize macroalgal-synthesized compounds. These enzymes are biological catalysts which have specific functional properties for biotechnological applications. Here, we raise awareness on the set of enzyme categories produced by the *Bacillus altidudinis* strain 19_A, isolated from the marine macroalga *Ulva lactuca,* as revealed by the analysis of its complete genome sequence. The genome of *B. altitudinis* strain 19_A is ~3.7 Mb long, has a G + C content of 41.2 %, and contains a total of 3,967 protein-coding genes. Our predictive analysis revealed that these genes encode proteases, lipases, esterases, and enzymes involved in the synthesis, degradation, and modification of carbohydrates. This enzyme repertoire may have promising biotechnological and industrial applications.
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Marine macroalgae establish mutualistic interactions with a diverse group of bacteria living on their surfaces (i.e. epiphytic bacteria) [1]. These interactions rely on the algal supply of organic substances for bacterial growth in exchange for secondary metabolites of bacterial origin important for algal spore release and settlement [2]. The environment on the macroalgal surface that supports the growth of their associated epiphytic bacteria constitutes an ideal source of multiple bacterial enzymes and bioactive compounds [1].

Macroalgae of the species *Ulva lactuca* and their most common bacterial epiphyte species, belonging to the gram-positive genus *Bacillus* [3], offer the possibility to investigate the array of bacterial enzymes that bring about their mutualistic relationship [1]. Furthermore, bacteria of the genus *Bacillus* are of interest because their enzymes have halophilic, thermophilic, and barophilic properties, and exhibit organic solvent tolerance [4]. *Bacillus* sp. produces a large variety of extracellular enzymes such as amylases, proteases, endoglucanases, and lipases with numerous industrial applications [5].

Macroalgal-associated bacteria harbor enzymes for the degradation of polysaccharide components of the macroalgal cell [1]. Based on their amino-acid sequence similarity, these enzymes are classified into families in the Carbohydrate-active enzymes database (CAZy) [6]. A *Bacillus* strain producing lipases, cellulases, and siderophores was recently isolated from the green macroalga *Ulva lactuca* [7]. In the present study, we raise awareness on the set of functional categories of the enzymes produced by the *Bacillus* strain 19_A, as revealed by the analysis of its complete genome sequence.

The genomic DNA of the strain 19_A was extracted using the ZR Soil Microbe DNA MicroPrep™ (Zymo Research) according to the manufacturer's instructions. Purity and concentration of the DNA were assessed using a Qubit® 2.0 Fluorometer (Thermo Scientific) and agarose gel electrophoresis. Sequencing libraries were prepared with the Illumina Nextera XT DNA library kit followed by paired-end sequencing on an Illumina NextSeq platform. A total of 13,695,819 paired-end reads were generated and processed with the Trimmomatic v0.38 software [8]. After discarding low-quality reads, the remaining 12,822,692 paired-end reads were assembled "*de novo*" with SPAdes v3.13.1 [9]. The obtained sequence coverage was 175×. The complete genome of the strain 19_A consisted of one circular chromosome of 3,730,920 bp with an average G + C content of 41,2 % (Table 1 and Fig. 1).

A phylogenetic tree based on 16S rRNA sequences showed that the strain 19_A forms a distinct branch together with *Bacillus altitudinis* SCU11 (Fig. 2). Their relatedness was also validated via two genome-to-genome sequence comparison approaches: the average nucleotide identity (ANI) analysis using the EzGenome ANI web resource (http://www.ezbiocloud.net/ezgenome/ani) and digital DNA-DNA hybridization (dDDH) with a genome-to-genome distance calculator (http://ggdc.dsmz.de/ggdc.php).

The assignment of strain 19_A to a species of genus *Bacillus* was confirmed based on the 95–96 % ANI and 70 % dDDH cut-offs [12].

* Corresponding author.
E-mail address: jose.montana@javeriana.edu.co (J.S. Montaña Lara).

https://doi.org/10.1016/j.btre.2021.e00634
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Table 1
Genome features of *Bacillus altitudinis* strain 19_A.

Attributes	Values
Genome size (bp)	3,730,920
G + C content (%)	41.2
Contigs	48
Protein-coding genes (CDS)	3,967
rRNA	4
tRNAs	44

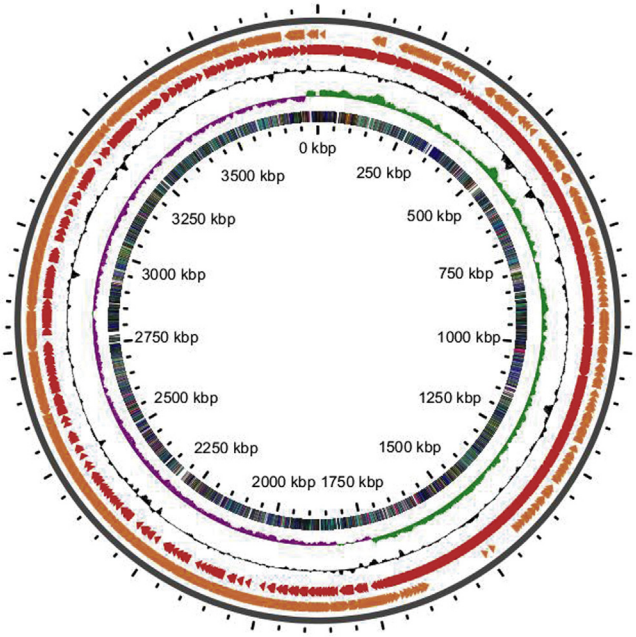

Fig. 1. Map of the *Bacillus altitudinis* strain 19_A genome. Ticked inner and outer most circles serve as size references. In-between and going inwards, circles 1 and 2 display the CDCs on the forward strand (orange) and reverse strand (red), respectively. Circle 3 displays the GC percentage plot. Circle 4 displays the GC skew (purple above average, green below average). Circle 5 displays the COG categories. The genome map was made using GView Server [10].

The ANI value for the comparison between the genomes of 19_A and *Bacillus altitudinis* was 98.49 % and their dDDH value was 86.60 %. According to this analysis, strain 19_A belongs to the species *Bacillus altitudinis*.

A total of 3,967 protein-coding genes were predicted using Prodigal v2.6.1 [13] and then annotated with RAST [14]. Furthermore, 44 tRNA and four rRNA genes were predicted with the online tools tRNAscan-SE v2.0 [15] and RNAmmer v1.2 for rRNA [16], respectively (Table 1 and Fig. 1).

The RAST annotation server predicted gene clusters coding for proteases (10 genes), lipases (2 genes), esterases (7 genes), and siderophores (21 genes). Macroalgae-associated microorganisms are known to produce diverse hydrolytic enzymes and bioactive compounds as they interact with their hosts and the marine environment (Table 2). Our analysis also identified genes for the protection from reactive oxygen species (13 genes) and genes responsible for choline and betaine uptake and biosynthesis (11 genes) in osmotic stress conditions.

Gene functional categories according the cluster of orthologous groups (COG) were determined with the online resource WebMGA [30]. The major COG functional categories found were replication; recombination and repair (L); translation; ribosomal structure and biogenesis (J); energy production and conversion (C); posttranslational modification; protein turnover; chaperones (O); intracellular trafficking; secretion and vesicular transport (U); inorganic ion transport and metabolism (I); and coenzyme transport and metabolism (H) (Table 3).

The enzymes likely involved in the degradation of macroalgal polysaccharides and the synthesis, degradation, and modification of carbohydrates (CAZymes) were analyzed with the server dbCAN [31]. The CAZymes analysis of the *B. altitudinis* genome revealed the presence of 15 genes for carbohydrate-binding modules (CMB), 26 genes for carbohydrate esterases (CE), 38 genes for glycoside hydrolases (GH), 30 genes for glycosyltransferases (GT), and 2 genes for polysaccharide lyases (PL).

Macroalgae are in large part composed of polysaccharides, as constituents of their extra- and intracellular matrices, cell walls, and storage compounds. Hence, the capability to degrade such polysaccharides constitutes an important trait of marine macroalgae-associated heterotrophic bacteria. The CAZyme profile of *B. altitudinis* strain 19_A is strongly indicative of an alga-associated lifestyle since it features CAZymes for the breakdown of many known polysaccharides specific to marine algae. CAZymes typically

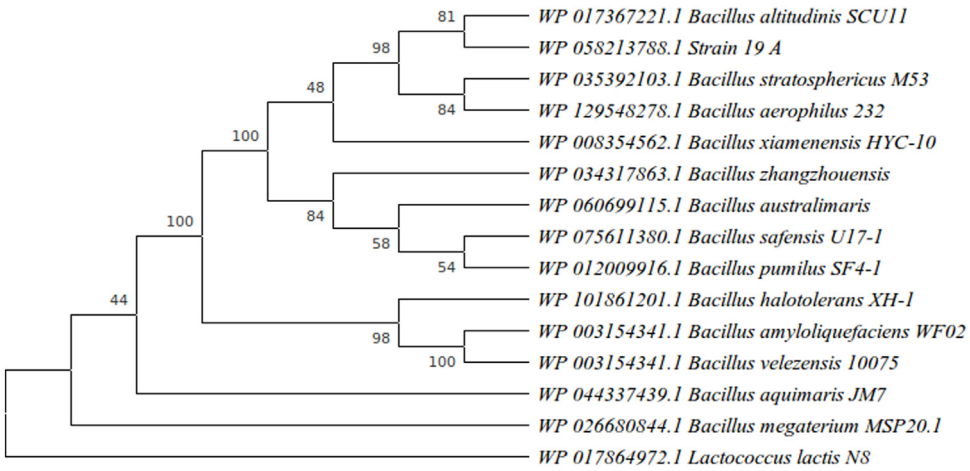

Fig. 2. Phylogenetic tree derived from 16S rRNA gene sequence data. Analyses were conducted in MEGA7 [11] and the distances were computed using the Maximum Likelihood method. A bootstrap test was performed on the clusters in 1000 replicates. Bootstrap values are displayed as percentages on their relative branches. The 16S rRNA sequence of *Lactococcus lactis* was employed as the outgroup.

Table 2
Characteristics of cultivable bacteria isolated from marine macroalgae tested for enzymatic activities and bioactive compounds. Bold font highlights *Bacillus altitudinis* strain 19_A.

Strain	Macroalgal Source	Activity	Reference
Bacillus	*Laurencia papillosa*	Antibacterial, antioxidant, antidiabetic, and anticancer	[17]
Roseobacter, Erythrobacter, Ruegeria, Epibacterium	*Halymenia floresii*	Quorum sensing	[18]
Vibrio, Bacillus, Pseudomonas	*Ulva lactuca*	Agarase, carrageenase, amylase, protease and cellulase	[19]
Epibacterium	*Ulva australis*	Antimicrobial	[20]
Strain 19_A	***Ulva lactuca***	**Cellulase, lipase and siderophores production**	[7]
Alcanivorax	*Ulva lactuca*	Antimicrobial	[21]
Bacillus	*Ulva rigida*	Antimicrobial	[22]
Paracoccus, Rhodobacteraceae	*Fucus spiralis*	Siderophores production	[23]
Vibrio, Halomonas	*Ulva* spp.	Ulvan lyase, Cellulase	[24]
Pseudomonas	*Padina tetrastromatica*	Antibacterial	[25]
Pseudoalteromonas, Vibrio, Shewanella	*Ulva lactuca*	Antibacterial and antidiatom	[26]
Bacillus	*Ulva lactuca*	Cellulase	[27]
Vibrio	*Ulva reticulata*	Antifouling	[28]
Pseudoalteromonas	*Ulva lactuca*	Antifouling	[29]

Table 3
Number of genes per cluster of orthologous groups (COG) functional categories in the *Bacillus altitudinis* strain 19_A genome.

COG	Functional category	Number of genes
J	Translation, ribosomal structure and biogenesis	207
A	RNA processing and modification	25
K	Transcription	44
L	Replication, recombination and repair	220
B	Chromatin structure and dynamics	19
D	Cell cycle control, cell division, chromosome partitioning	60
V	Defense mechanisms	10
T	Signal transduction mechanisms	80
M	Cell wall/membrane/envelope biogenesis	103
N	Cell motility	67
Z	Cytoskeleton	12
U	Intracellular trafficking, secretion, and vesicular transport	154
O	Posttranslational modification, protein turnover, chaperones	164
C	Energy production and conversion	188
G	Carbohydrate transport and metabolism	112
E	Amino acid transport and metabolism	120
F	Nucleotide transport and metabolism	77
H	Coenzyme transport and metabolism	140
I	Lipid transport and metabolism	56
P	Inorganic ion transport and metabolism	149
Q	Secondary metabolites biosynthesis, transport and catabolism	48
R	General function prediction only	595
S	Function unknown	1,317
	Total	3,967

account for up to 2 % of the genes in most bacterial genomes [32]. The genome of the *B. altitudinis* strain 19_A features 2.8 % CAZymes, which reflects the specialization of this bacterium to degrade macroalgal macromolecules.

Thanks to the CAZyme analysis, we identified pectin-hydrolyzing enzymes. Pectin is one of the components of cell walls in terrestrial plants and has also been found in some species of macroalgae such as *Ulva* [33]. The genome of the *B. altitudinis* strain 19_A revealed the presence of a coding gene for a Pectate lyase (PL1_BA), which catalyzes the cleavage of pectate via a β-elimination reaction and generates 4,5-unsaturated oligogalacturonate [34]. Pectate lyases are used in the food and paper industries, textile and plant fiber processing, oil extraction, and industrial wastewater treatment [35].

Pectate lyase amino acid sequences from various *Bacillus* species were downloaded from the NCBI repository (https://www.ncbi.nlm.nih.gov/) for multiple protein sequence alignment using the ClustalW (http://www.ebi.ac.uk/clustalW/) and BLAST programs (http://www.ncbi.nlm.nih.gov/BLAST). The predicted amino acid sequence of PL1_BA showed the highest similarity (98.8 % identity) with a pectate lyase from *Bacillus pumilus* DKS1 (GenBank No. ACD11362), a thermostable and alkaline extracellular enzyme [36]. In addition, PL1_BA showed high similarity to other pectate lyases from *Bacillus* sp. KSM-P103 (GenBank No. BAA76885; [37]) and *Bacillus* sp. strain KSM-P7 (GenBank No. BAA76884; [38]) with 98 % and 87.5 % identity, respectively.

The analysis of the sequence alignment of PL1_BA and other pectate lyases showed that residues R235 and R240/K206 of PL1_BA are conserved and are likely to constitute the catalytic center of the enzyme (Fig. 3). Furthermore, the three aspartate residues D152, D182 and D186 are also conserved and are probably the Ca^{+2} binding sites required for the enzymatic activity [34]. The PL1_BA protein also has a typical amino-terminal signal sequence with 30 amino acids (Fig. 3), which was identified using the SignalP 4.1 server (http://www.cbs.dtu.dk/services/SignalP/).

Our genome sequencing effort and its analysis have provided a view into the composition of the enzyme repertoire of the epiphytic bacteria *B. altitudinis* strain 19_A, isolated from the

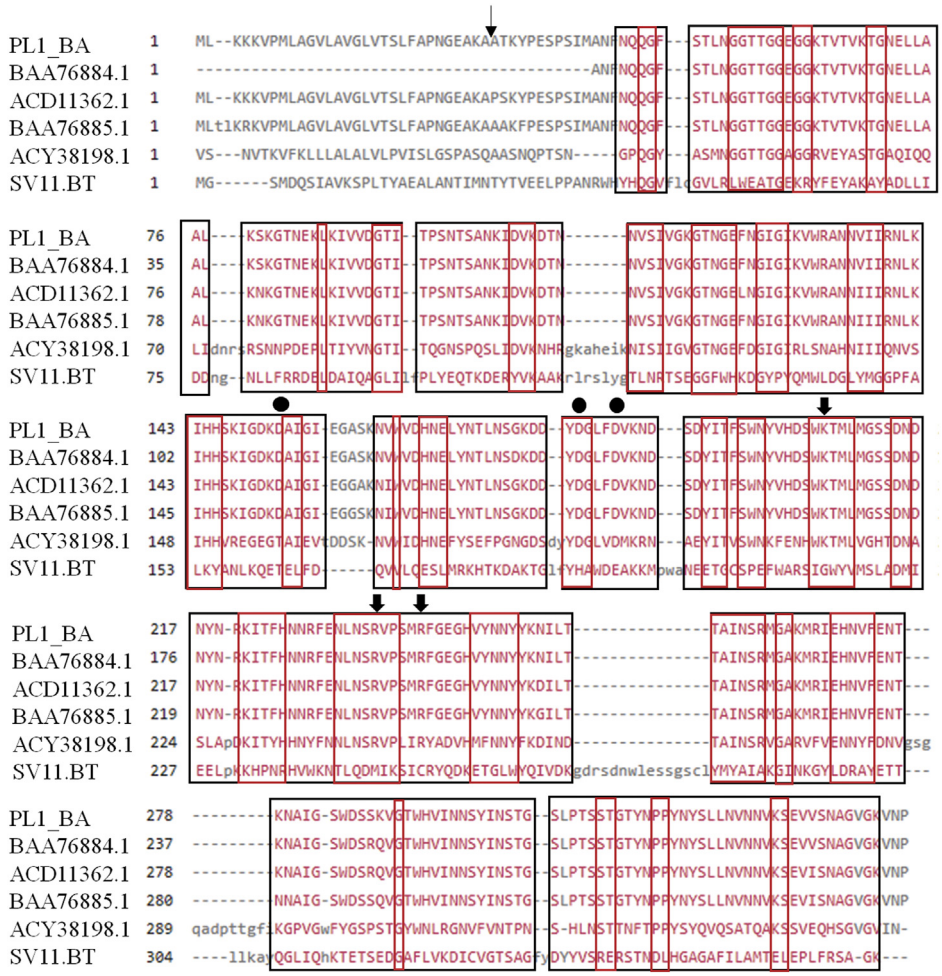

Fig. 3. Multiple amino acid sequence alignment of PL1_BA and other pectate lyases. The pectate lyases used were: BAA76884.1 from *Bacillus* sp. strain KSM-P7, ACD11362.1 from *B. pumilus* DKS1, BAA76885.1 from *Bacillus* sp. KSM-P103, ACY38198.1 from *Bacillus* sp. N16-5, and SV11.BT from *Bacillus tequilensis* SV11. Identical residues are within frames in red. ⬇ marks the essential catalytic bases. ● marks the position of the three conserved aspartate residues for Ca^{2+} binding. The slender arrow shows the cleavage site of the signal peptide of PL1_BA.

marine macroalga *Ulva lactuca*. The results show that this strain is a source of enzymes with a potential application in biotechnological and industrial processes.

Nucleotide sequence and strain accession numbers

The genome sequence of *Bacillus altitudinis* strain 19_A was deposited at DDBJ/ENA/GenBank under accession number NZ_CABEHU000000000.1. Strain 19_A is currently available from the Collection of Microorganisms of the Pontificia Universidad Javeriana (CMPUJ) under strain code CMPUJ 454.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was funded by the Corporation Center of Excellence in Marine Sciences (CEMarin) (Call No. 8, 2016). ANLA (Autoridad Nacional de Licencias Ambientales) and Ministerio de Ambiente y Desarrollo Sostenible granted permission to collect samples and perform this research (Resolución No. 0255 of 14/03/2014, Contrato de Acceso a Recursos Genéticos y sus Productos Derivados No. 121 of 22/01/2016, OTROSÍ No. 22). The authors would like to thank the Pontificia Universidad Javeriana for their support to the postdoctoral stay (call 2018).

References

[1] S. Egan, T. Harder, C. Burke, P. Steinberg, S. Kjelleberg, T. Thomas, The seaweed holobiont: understanding seaweed–bacteria interactions, FEMS Microbiol. Rev. 37 (2013) 462–476.
[2] J. Hollants, F. Leliaert, O. De Clerk, A. Willems, What we can learn from sushi: a review on seaweed-bacterial associations, FEMS Microbiol. Ecol. 83 (2013) 1–16, doi:http://dx.doi.org/10.1111/j.1574-6941.2012.01446.x.
[3] Y. Xiong, R. Yang, X. Sun, H. Yang, H. Chen, Effect of the epiphytic bacterium *Bacillus* sp. WPySW2 on the metabolism of *Pyropia haitanesis*, J. Appl. Phycol. 30 (2) (2018) 1225–1237, doi:http://dx.doi.org/10.1007/s10811-017-1279-z.
[4] P. Esakkiraj, C. Antonyraj, B. Meleppat, D. Ankaiah, R. Ayyanna, S. Ahamed, V. Arul, Molecular characterization and application of lipase from *Bacillus* sp. PU1 and investigation of structural changes based on pH and temperature using MD simulation, Int. J. Biol. Macromol. 103 (2017) 47–56, doi:http://dx.doi.org/10.1016/j.ijbiomac.2017.04.111.
[5] R. Vaikundamoorthy, R. Rajendran, A. Selvaraju, K. Moorthy, S. Perumal, Development of thermostable amylase enzyme from *Bacillus cereus* for potential antibiofilm activity, Bioorg. Chem. 77 (2018) 494–506, doi:http://dx.doi.org/10.1016/j.bioorg.2018.02.014.
[6] V. Lombard, T. Bernard, C. Rancurel, H. Brumer, P.M. Coutinho, B. Henrissat, A hierarchical classification of polysaccharide lyases for glycogenomics, Biochem. J. 432 (3) (2010) 437–444, doi:http://dx.doi.org/10.1042/bj20101185.
[7] N. Comba, M.L. Ramírez, L. López, D. Montoya, Production of enzymes and siderophores by epiphytic bacteria isolated from the marine macroalga *Ulva*

lactuca, Aquatic Biol. 27 (2018) 107–118, doi:http://dx.doi.org/10.3354/ab00700.
[8] A.M. Bolger, M. Lohse, B. Usadel, Trimmomatic: a flexible trimmer for Illumina sequence data, Bioinformatics 30 (15) (2014) 2114–2120, doi:http://dx.doi.org/10.1093/bioinformatics/btu170.
[9] A. Bankevich, S. Nurk, D. Antipov, A.A. Gurevich, M. Dvorkin, A.S. Kulikov, V.M. Lesin, S.I. Nikolenko, S. Pham, A.D. Prjibelski, A.V. Pyshkin, SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing, J. Comput. Biol. 19 (5) (2012) 455–477, doi:http://dx.doi.org/10.1089/cmb.2012.0021.
[10] A. Petkau, M. Stuart-Edwards, P. Stothard, G. Van Domselaar, Interactive microbial genome visualization with GView, Bioinformatics 26 (2010) 3125–3126.
[11] S. Kumar, G. Stecher, K. Tamur, MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets, Mol. Biol. Evol. 33 (7) (2016) 1870–1874, doi:http://dx.doi.org/10.1093/molbev/msw054.
[12] M. Richter, R. Rosselló-Móra, Shifting the genomic gold standard for the prokaryotic species definition, PNAS 106 (2009) 19126–19131.
[13] D. Hyatt, G. Chen, P. LoCascio, M. Land, F. Larimer, L. Hauser, Prodigal: prokaryotic gene recognition and translation initiation site identification, BMC Bioinformatics 11 (119) (2010) 1–11, doi:http://dx.doi.org/10.1186/1471-2105-11-119.
[14] R.K. Aziz, D. Bartels, A.A. Best, M. DeJongh, T. Disz, R.A. Edwards, K. Formsma, S. Gerdes, E.M. Glass, M. Kubal, F. Meyer, G.J. Olsen, R. Olson, A.L. Osterman, R.A. Overbeek, L.K. McNeil, D. Paarmann, T. Paczian, B. Parrello, G.D. Pusch, C. Reich, R. Stevens, O. Vassieva, V. Vonstein, A. Wilke, O. Zagnitko, The RAST Server: rapid annotations using subsystems technology, BMC Genomics 9 (75) (2008) 1–15, doi:http://dx.doi.org/10.1186/1471-2164-9-75.
[15] T.M. Lowe, P.P. Chan, tRNAscan-SE on-line: search and contextual analysis of transfer RNA Genes, Nucl. Acids Res. 44 (2016) W54–57.
[16] K. Lagesen, O. Hallin, E.A. Rodland, H. Staerfeldt, T. Rognes, D.W. Ussery, RNAmmer: consistent and rapid annotation of ribosomal RNA gene, Nucl. Acids Res. 35 (9) (2007) 3100–3108, doi:http://dx.doi.org/10.1093/nar/gkm160.
[17] A. Francis, K. Chakraborty, Marine macroalga-associated heterotroph *Bacillus velezensis* as prospective therapeutic agent, Arch. Microbiol. 203 (2021) 1671–1682, doi:http://dx.doi.org/10.1007/s00203-020-02169-3.
[18] S. Malik, A. Bazire, A. Gamboa, G. Bedoux, D. Robledo, J. García, N. Bourgougnon, Screening of surface-associated bacteria from the Mexican red alga *Halymenia floressi* for quorum sensing activity, Microbiology 89 (6) (2020) 778–788, doi:http://dx.doi.org/10.1134/S0026261720060132.
[19] M. Naik, D. Naik, L. Charya, S. Mujawar, D. Vaingankar, Application of marine bacteria associated with seaweed, *Ulva lactuca*, for degradation of algal waste, Proc. Natl. Acad. Sci. India Sect. B Biol. Sci. 89 (4) (2019) 1153–1160, doi:http://dx.doi.org/10.1007/s40011-018-1034-5.
[20] S. Breider, S. Sehar, M. Berger, T. Thomas, T. Brinkhoff, S. Egan, Genome sequence of *Epibacterium ulvae* strain DSM 24752T, an indigoidine-producing, macroalga-associated member of the marine Roseobacter group, Environ. Microbiome 14 (4) (2019) 1–8, doi:http://dx.doi.org/10.1186/s40793-019-0343-5.
[21] P. Karthick, R. Mohanraju, Antimicrobial potential of epiphytic bacteria associated with seaweeds of Little Andaman, India, Front. Microbiol. 9 (611) (2018) 1–11, doi:http://dx.doi.org/10.3389/fmicb.2018.00611.
[22] A. Ismail, L. Ktari, M. Ahmed, H. Bolhuis, B. Bouhaouala, L. Stal, A. Boudabbous, M. Bour, Heterotrophic bacteria associated with the green alga *Ulva rigida*: identification and antimicrobial potential, J. Appl. Phycol. 30 (2018) 2883–2899, doi:http://dx.doi.org/10.1007/s10811-018-1454-x.
[23] M. Dogs, B. Wemheuer, L. Wolter, N. Bergen, R. Daniel, M. Simon, T. Brinkhoff, Rhodobacteraceae on the marine brown alga *Fucus spiralis* are abundant and show physiological adaptation to an epiphytic lifestyle, Syst. Appl. Microbiol. 40 (6) (2017) 370–382, doi:http://dx.doi.org/10.1016/j.syapm.2017.05.006.
[24] V. Rodrigues, L. Onime, S. Huws, A. Odaneth, A. Lali, Diversity of ulvan and cellulose depolymerizing bacteria associated with the green macroalgae *Ulva* spp, J. Appl. Biotechnol. Bioeng. 2 (4) (2017) 136–142, doi:http://dx.doi.org/10.15406/jabb.2017.02.00037.
[25] A. Ravisankar, M. Gnanambal, L. Sundaram, A newly isolated *Pseudomonas* sp., epibiotic on the seaweed, *Padina tetrastromatica*, off southeastern coast of India, reveals antibacterial action, Appl. Biochem. Biotechnol. 171 (2013) 1968–1985, doi:http://dx.doi.org/10.1007/s12010-013-0473-y.
[26] V. Kumar, D. Rao, T. Thomas, S. Kjelleberg, S. Egan, Antidiatom and antibacterial activity of epiphytic bacteria isolated from *Ulva lactuca* in tropical waters, World J. Microbiol. Biotechnol. 27 (2011) 1543–1549, doi:http://dx.doi.org/10.1007/s11274-010-0606-1.
[27] N. Trivedi, V. Gupta, M. Kumar, P. Kumari, C. Reddy, B. Jha, An alkali-halotolerant cellulase from *Bacillus flexus* isolated from green seaweed *Ulva lactuca*, Carbohydr. Polym. 83 (2) (2011) 891–897, doi:http://dx.doi.org/10.1016/j.carbpol.2010.08.069.
[28] T. Harder, S. Dobretsov, P. Qian, Waterborne polar macromolecules act as algal antifoulants in the seaweed *Ulva reticulata*, Mar. Ecol. Prog. Ser. 274 (2004) 133–141.
[29] S. Egan, T. Thomas, C. Holmström, S. Kjelleberg, Phylogenetic relationship and antifouling activity of bacterial epiphytes from the marine alga *Ulva lactuca*, Environ. Microbiol. 2 (2000) 343–347.
[30] S. Wu, Z. Zhu, L. Fu, B. Niu, W. Li, WebMGA: a customizable web server for fast metagenomic sequence analysis, BMC Genomics 12 (2011) 444.
[31] Y. Yin, X. Mao, J. Yang, X. Chen, F. Mao, Y. Xu, DbCAN: a web resource for automated carbohydrate-active enzyme annotation, Nucleic Acids Res. 40 (2012) W445–W451, doi:http://dx.doi.org/10.1093/nar/gks479.
[32] A. Mann, R. Hahnke, S. Huang, J. Werner, P. Xing, T. Barbeyron, B. Huettel, K. Stüber, R. Reinhardt, J. Harder, F. Glöckner, R. Amann, H. Teeling, The genome of the alga-associated marine flavobacterium *Formosa agariphila* KMM 3901T reveals a broad potential for degrading of algal polysaccharides, Appl. Environ. Microbiol. 79 (21) (2013) 6813–6822, doi:http://dx.doi.org/10.1128/AEM.01937-13.
[33] A. Holzinger, K. Herburger, F. Kaplan, L. Lewis, Desiccation tolerance in the chlorophyte green alga *Ulva compressa*: does cell architecture contribute to ecological success? Plant 242 (2015) 477–492, doi:http://dx.doi.org/10.1007/s00425-015-2292-6.
[34] C. Zhou, Y. Xue, Y. Ma, Characterization and overproduction of a thermo-alkaline pectate lyase from alkaliphilic *Bacillus licheniformis* with potential in ramie degumming, Process Biochem. 54 (2017) 49–58, doi:http://dx.doi.org/10.1016/j.procbio.2017.01.010.
[35] R. Jayani, S. Saxena, R. Gupta, Microbial pectinolytic enzymes: a review, Process Biochem. 40 (9) (2005) 2931–2944, doi:http://dx.doi.org/10.1016/j.procbio.2005.03.026.
[36] S. Basu, A. Ghosh, A. Bera, M. Saha, D. Chattopadhyay, K. Chakrabarti, Thermodynamic characterization of a highly thermoactive extracellular pectate lyase from a new isolate *Bacillus pumilus* DSK1, Bioresour. Technol. 99 (17) (2008) 8088–8094, doi:http://dx.doi.org/10.1016/j.biortech.2008.03.032.
[37] Y. Hatada, N. Higaki, K. Saito, A. Ogawa, K. Sawada, T. Ozawa, Y. Hakamada, T. Kobayashi, S. Ito, Cloning and sequencing of a high-alkaline pectate lyase gene from an alkaliphilic *Bacillus* isolate, Biosci. Biotechnol. Biochem. 63 (6) (1999) 998–1005, doi:http://dx.doi.org/10.1271/bbb.63.998.
[38] T. Kobayashi, Y. Hatada, N. Higaki, D. Lusterio, T. Ozawa, K. Koike, S. Kawai, S. Ito, Enzymatic properties and deduced amino acid sequence of a high-alkaline pectate lyase from an alkaliphilic *Bacillus* isolate, Biochim. Biophys. Acta 1427 (2) (1999) 145–154, doi:http://dx.doi.org/10.1016/s0304-4165(99)00017-3.